ANALYSIS OF CHROMOSOMAL DNA CONTENT IN PACIFIC RED ABALONE *Haliotis rufescens* BY FLUORESCENCE IMAGE ANALYSIS

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The Pacific red abalone Haliotis rufescens (Swainson, 1822) is a marine gastropod inhabiting along the Pacific coast from Southern Oregon – Northern California to the Baja California Peninsula in Mexico.

Together with banding and *FISH* techniques, flow cytometry has been used for the analysis of DNA contents. However application of flow cytometry and chromosomal sorting had been used mainly in plants and mammals. The implementing of these techniques in molluscs has been delayed by difficulties in obtaining cellular line culture.

The fluorescence fading and chromosome imaging method were jointly used to analyze chromosomal DNA contents (haploid set) in Pacific red abalone. The fluorescence intensity was measured in *H. rufescens* chromosomes (2n=36) stained with DAPI. The fluorescence of each chromosomal type was recorded using color digital images of 24 bits. This kind of images allowed quantifies the fluorescence intensity by means of 256 levels of brightness.

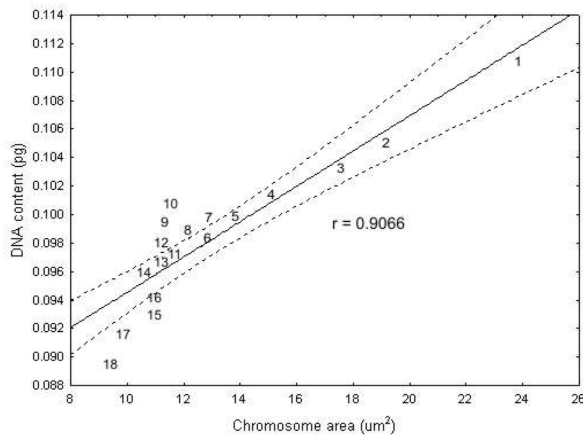

Figure 1. Correlation between chromosomal DNA content and chromosome area. The numbers inside represent the chromosome type. Discontinuous plotted line represents calculated standard error.

Their DNA contents were related with chromosomal sizes ($p<0.001$) (Figure 1), and the DNA values found showed a range from 0.1106 ± 0.0045 pg (chromosome # 1) to 0.0890 ± 0.0060 pg (chromosome # 18). The genome size calculated for *H. rufescens* by sum of all chromosomal DNA contents was 1.7717 ± 0.005 pg (Table 1). This study describes an alternative imaging method for analyzing chromosome morphology and chromosomal DNA contents in molluscan chromosomes.

Table 1. Individual chromosome DNA content in Haliotis rufescens and its conversion into Mega base pairs (Mbp).

Chromosome	Mean DNA content (pg)	Standard deviation (pg)	Mbp*
1	0.1106	0.0045	108,201
2	0.1051	0.0098	102,752
3	0.1035	0.0053	101,175
4	0.1010	0.0093	98,824
5	0.0999	0.0077	97,655
6	0.0976	0.0072	95,490
7	0.0997	0.0089	97,474
8	0.0982	0.0099	96,087
9	0.0994	0.0063	97,243
10	0.1006	0.0094	98,393
11	0.0975	0.0104	95,319
12	0.0979	0.0085	95,757
13	0.0969	0.0075	94,784
14	0.0958	0.0087	93,690
15	0.0934	0.0090	91,355
16	0.0939	0.0076	91,825
17	0.0917	0.0077	89,640
18	0.0890	0.0060	87,056
Total	1.7717	0.0050	1,732,720

* Number of base pairs = mass in pg x 0.978×10^9 (Dolezel et al. 2003).

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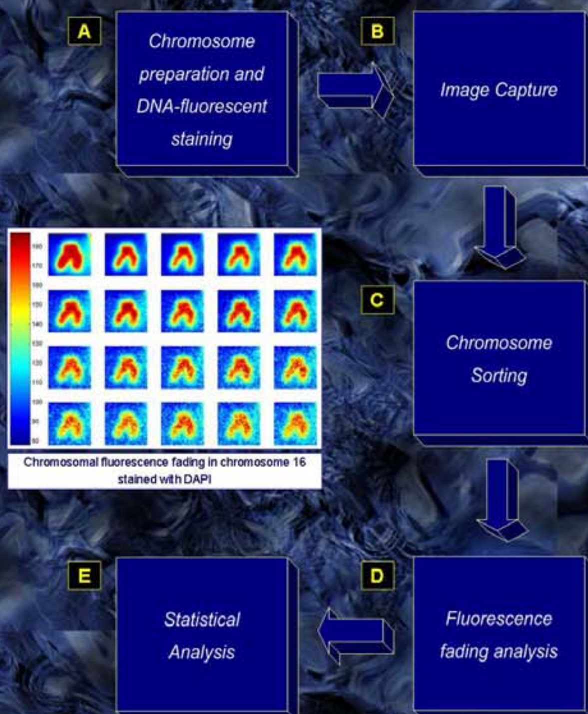
INTRODUCTION

The Pacific red abalone *Haliotis rufescens* is a marine gastropod inhabiting rocky substrates together with kelp along the Pacific coast from Southern Oregon – Northern California in the United States to the Baja California Peninsula in Mexico. Abalones are economically important marine gastropods that reach moderate to high prices in the world market. Aquaculture production of red abalone began during the last decade in Mexico. The whole life cycle is managed under controlled conditions. Because of its economic importance, several studies of ploidy manipulation have been carried out to enhance the production of different abalone species. Modern cytogenetic methods such as chromosome banding and fluorescent *in situ* hybridization (FISH) proved to be very useful to understand the genomic organization of numerous vertebrate species. In this context, to ascertain the cytogenetic characteristics of *H. rufescens*, the karyotype of this species (2n=36) has been reported with eight metacentric, eight submetacentric, one submetacentric / metacentric and one subtelocentric chromosome pair. Together with banding and FISH techniques, flow cytometry has been used for the analysis of DNA contents of genomes, specific chromosomes, and chromosomal karyotyping. However, the application of flow cytometry and sorting (flow cytogenetics) had been used mainly in plant and human genome. The implementing of these techniques in molluscs have been delayed by difficulties in obtaining cellular line culture, therefore preparation of suspensions of intact chromosomes and discrimination of individual chromosome type by cytometry remains nowadays in stand by. Recently, image analysis methods have been introduced in cytogenetic research. It has been shown that the genome size data obtained by image analysis methods are comparable to flow cytometry data. A new method for the estimation of cell DNA content using fluorescence fading has been developed. The method consisting on the measure of the fluorescence intensity in DAPI-stained nuclei of several species, where the estimation of the area under the curve (Integral fading) during a fading period was related with the genome size.

METHODOLOGY



Chromosomal fluorescence fading in chromosome 16 stained with DAPI

ACKNOWLEDGMENTS

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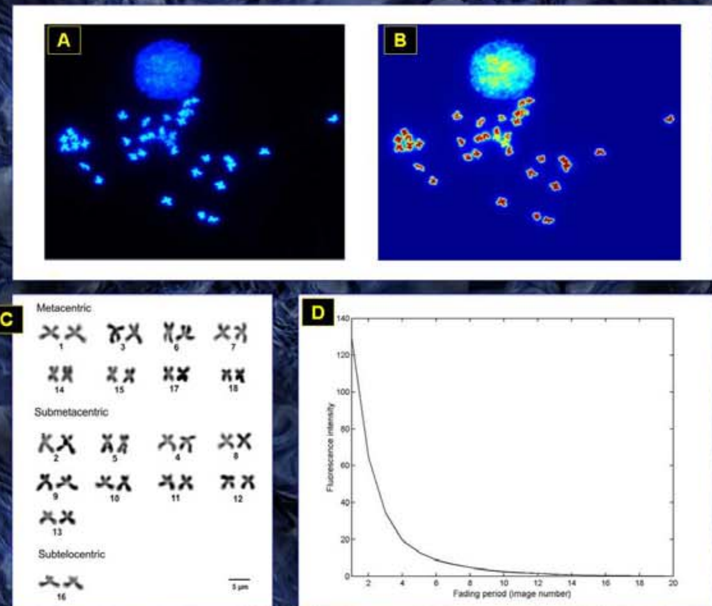
RESULTS



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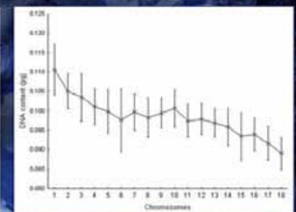

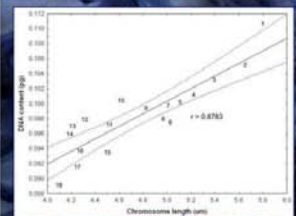

DISCUSSION

1. The cytogenetic studies in invertebrates like molluscs are particularly problematic mainly due to their smaller chromosome size and degree of condensation.
2. Flow karyotyping in molluscs showed problems due to difficulty in obtaining pure cellular line culture. The present study shows an alternative method to estimate the chromosomal DNA contents by fluorescence image analysis. Our results showed that there is a positive correlation with their chromosome sizes ($p<0.001$). The correlation value determined for chromosome area was of $r = 0.9066$ and chromosome length was of $r = 0.8783$. However, it was difficult to sort chromosomes intermediate sizes using chromosomal DNA contents. In this context, statistical analysis showed differences only among extreme chromosomes (1-4 and 13-18). This study, showed largest chromosomal DNA variability in chromosomes 2, 4, 8, 11 and 15.
3. In reference to fluorescence fading method, this technique is based on the fluorescent decay lifetime when the fluorochrome is exposed to excitation light. The photochemical process underlying the fluorescence decay of DAPI has not yet been fully explained, although theories suggesting the involvement of oxygen, triplet states and protein denaturalization have been proposed.
4. Our proposed method only used PBS like mounting medium, therefore the photochemical process is not modified. In this study we estimated the individual DNA content for each chromosome types and the total DNA content or genome size refers to the haploid amount of DNA. Our data show a genome size for *Haliotis rufescens* of 1.7717 ± 0.0050 pg. Hinigardner (1974) using classical techniques reported for this species a genome size of 1.8 pg.